PRODUCT INFORMATION SHEET
Monoclonal antibodies detecting human antigens

Anti-TdT

FITC	RUO	REF	IQP-149F	10 tests
FITC	RUO	REF	IQP-150F	50 tests

RUO ***For Research Use Only***

Description

Clone HT-6

Isotype mouse IgG1

Specificity Clone HT-6 produces mouse IgG1 immunoglobulins directed against the human TdT antigen. TdT is a 58 kD protein. Anti-TdT antibodies are reactive with approx. less than 10% of bone marrow cells, while less than 0.1% of TdT-positive cells are found in normal blood samples.

Antigen distribution

Terminal deoxynucleotidyl transferase (TdT) is an enzyme which is involved in DNA polymerization and is localized in the nucleus of hematopoietic cells, e.g. prothymocytes, precursor T- and a subset of precursor B-cells. Clone HT-6 antibodies, can detect intracellular TdT by flow cytometric analysis. TdT is expressed in most cases of acute lymphatic leukemia (ALL), in a subset of chronic or acute myeloid leukemia (CML and AML respectively), acute non-lymphocytic leukemia (ANLL) and Non-Hodgkin's Lymphoma (NHL) [1,2]. Detection of nuclear expression of TdT by flow cytometry is a valuable technique in the characterization of leukemias and monitoring minimal residual leukemic cells.

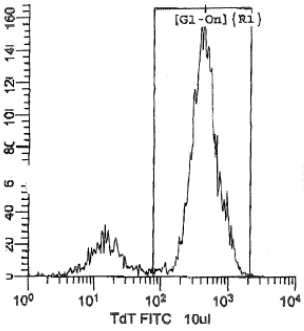
Applications Monoclonal antibodies anti-TdT-FITC, clone HT-6, can be applied in flow cytometry for analysis of blood or bone marrow samples. This reagent is not intended for use in TdT slide tests. Expression of TdT has been known for many years as a nuclear marker for hematopoietic cells [1]. The antigen, a DNA polymerase, is believed to be involved in rearrangement of T-cell receptor genes in early thymocytes, and in rearrangement of the immunoglobulin genes during B cell development. Both activities are performed in the nucleus and are lost during maturation of immune cells. Analysis by flow cytometry can be useful in detecting small numbers of leukemic cells, such as in minimal residual T cell ALL [3]. Furthermore, simultaneous analysis of cell surface antigens and nuclear TdT enables the reliable detection of as few as 0.02% of leukemic cells [4]. Double staining of T-ALL samples are often performed with anti-TdT in combination with CD1a, CD2, CD5, and CD7 antibodies. A useful three color flow cytometric detection method for analysis of leukemic cells, involves gating with CD45, and analysis of intracellular markers, such as TdT and as cell membrane marker e.g. CD19 in samples of precursor B-ALL [6].

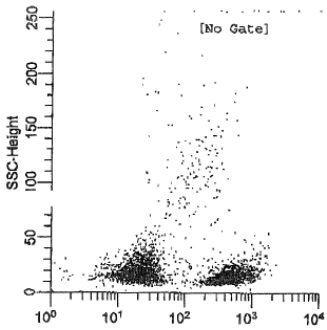
TdT is expressed in a subset of AML (approx. 15%), and may be indicative of a poor prognosis in a subgroup of patients [4,5]. Flow cytometric analysis of AML shows comparable results to microscopic procedures [5]. Comparison of microscopic and flow cytometric detection of TdT showed comparable results [5]. The sensitivity of the detection of myeloblasts could be enhanced to levels of approx. 0.4-0.5% of cells, using the technique of double staining. Monoclonal antibodies used for double staining of AML samples are e.g. anti-HLA-DR, CD13, CD33, CD34 and CD45 antibodies.

Usage All these reagents are effectively formulated for direct immunofluorescent staining of human tissue for flow cytometric analysis using 10 µL/10^6 leukocytes for singles and 20 µL/10^6 leukocytes in case of dual and triple combinations. Since applications vary, each investigator should titrate the reagent to obtain optimal results.

Representative Data

A preparation of FITC-conjugated HT-6 (anti-TdT) monoclonal antibodies was analyzed by flow cytometry using a bone marrow sample from a T-ALL patient. Direct staining was performed using 10 µl of monoclonal antibody preparation and 100 µl of Ficoll Isopaque purified bone marrow cells.





Limitations

1. Conjugates with brighter fluorochromes, like PE and APC, will have a greater separation than those with dyes like FITC and CyQ. When populations overlap, the percentage of positive cells using a selected marker can be affected by the choice of fluorescent label.
2. Use of monoclonal antibodies in patient treatment can interfere with antigen target recognition by this reagent. This should be taken into account when samples are analyzed from patients treated in this fashion. IQ Products has not characterized the effect of the presence of therapeutic antibodies on the performance of this reagent.
3. Reagents can be used in different combinations, therefore laboratories need to become familiar performance characteristics of each antibody in relation with the combined markers in normal and abnormal samples.
4. Reagent data performance is based on EDTA-treated blood. Reagent performance can be affected by the use of other anticoagulants.

Reagents and materials required but not supplied

1. Flow cytometer
2. Flow cytometry disposable 12 x 75-mm capped polystyrene test tubes
3. Micropipette with disposable tips
4. Vortex mixer
5. Centrifuge
6. IQ Lyse - erythrocyte lysing solution (IQP-199)
7. IQ Starfiqs – fixation and permeabilization solution (IQP-200)
8. PBS (phosphate-buffered saline)
9. 1% paraformaldehyde solution in PBS (store at 2-8 °C in amber glass for up to 1 week)

Protocol for immuno-fluorescence staining of intracellular antigens

IQ Starfiqs is a fixation and permeabilization solution intended for preparation of blood leukocytes before flow cytometry analysis of intracellular antigens. **IQ Starfiqs** is a **ready to use** product, composed of two reagents used sequentially. The composition of both reagents is adjusted to ensure an optimum performance in flow cytometry analysis. Both reagents should be stored at 4 – 8 °C till the expiration period as indicated.

For optimal intracellular immunostaining and lysing of erythrocytes, **IQ Starfiqs** should be used following the complete procedure as indicated below (see protocol). **IQ Starfiqs** enables the detection of intracellular antigens such as CyCD3, CyCD22, TdT and MPO (myeloperoxidase). Results of analysis of blood samples for intracellular detection of MPO are shown below.
In addition, the application of **IQ Starfiqs** allows the simultaneous detection of cell surface antigens (see extended protocol **IQ Starfiqs**). It is important to use both reagents and not to mix with other products.
IQ Starfiqs is provided as a ready to use product, to minimize hands on time and the easy handling of samples.

Protocol IQ Starfiqs (staining of intracellular antigens)

- Add 100 µl EDTA treated whole blood (bone marrow sample, mononuclear cell suspension) to a reagent tube.
- Add 100 µl **IQ Starfiqs** fixation reagent (Reagent F).
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 100 µl of **IQ Starfiqs** permeabilization reagent (Reagent P).
- Add 10 µl of IQ Products antibody conjugate for single reagent, or 20 µl of antibody conjugate for dual reagent.
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline.

Extended Protocol IQ Starfiqs (staining of cell surface antigens and intracellular antigens)

- Add antibody conjugate to a reagent tube: 10 µl of antibody conjugate for single reagent directed against a cell surface antigen.
- Add 100 µl of EDTA- or Heparin-treated whole blood and mix well.
- Incubate for 15 minutes at room temperature in the dark.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove the supernatant.
- Add 100 µl **IQ Starfiqs** fixation reagent (Reagent F).
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove the supernatant and resuspend the cell pellet in 100 µl of **IQ Starfiqs** permeabilization reagent (Reagent P).
- Add 10 µl of IQ Products antibody conjugate for single reagent directed against an intracellular antigen.
- Incubate for 15 minutes at room temperature.
- Add 4 ml phosphate buffered saline pH 7.3 and centrifuge for 5 minutes at 300xg.
- Remove supernatant and resuspend the cell pellet in 200 µl of phosphate buffered saline.

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Handling and Storage

Antibodies are supplied either as 100 tests per vial (1 mL) for singles or 50 tests per vial (1 mL) for dual and triple combinations. They are supplied in 0.01 M sodium phosphate, 0.15 M NaCl; pH 6.9, 1.0% BSA, 0.1% sodiumazide (NaN_3). Store the vials at 2-8 °C. Monoclonal antibodies should be protected from prolonged exposure to light. Reagents are stable for the period shown on the vial label when stored properly

Warranty Products sold hereunder are warranted only to conform to the quantity and contents stated on the label at the time of delivery to the customer. There are no warranties, expressed or implied, which extend beyond the description on the label of the product. IQ Products is not liable for property damage, personal injury, or economic loss caused by the product

Characterization

To ensure consistently high-quality reagents, each batch of monoclonal antibody is tested for conformance with characteristics of a standard reagent. Representative flow cytometric data is included in this data sheet

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Warning All products contain sodiumazide. This chemical is poisonous and hazardous. Handling should be done by trained staff only

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References

1. Bollum, F.J. 1979. Blood, 54, 1203-1215
2. Slaper-Cortenbach, I.C.M. et al. 1988. Blood, 72, 1639-1644
3. Gore, S.D. et al.1990. Immunol. Methods,, 132, 275-286
4. Drach, J. et a l. 1991. Br. J. Hematol, 77, 37-42
5. Paiettta, E. et al., 1994. Cytometry 16 256-261
6. Horvatinovitch, J.M. et al. 1994. Cytometry, 18, 228-230

Explanation of used symbols

	Consult instructions for use
REF	Catalogue number
	Sufficient for
IVD	In Vitro Diagnostic medical device
	Caution, consult accompanying document
	Keep away from (sun)light
	Biological risks
	Temperature limitation (°C)
RUO	For Research Use Only
LOT	Batch code
	Use by yyyy-mm-dd
	Manufacturer
EC REP	Authorized Representative in the European Community
CE	Conformité Européenne (European Conformity)

		Label - tandem	**Ex -max (nm)**	**Em -max (nm)**
P	PURE	purified material	-	-
F	FITC	FITC	488	519
R	R-PE	PE	488, 532	578
C	CyQ	PE-Cy5.18	488, 532	667
A	APC		595, 633, 635, 647	660
PC	PerCP		488, 532	678
PCC	PerCP-Cy5.5		488, 532	695

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